DIVISION OF PRODUCT QUALITY CONTROL

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

ANNUAL REPORT

October 1, 1991 through September 30, 1992

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Division of Product Quality Control
Director's Summary
October 1, 1991 through September 30, 1992

Program Responsibilities

The primary functions of DPQC are to:

a) Conduct sterility, general safety, pyrogen and potency tests, as well as to perform safety, neurovirulence and potency tests utilizing nonhuman primates with biological products submitted either for release or in support of licensing actions.

b) Establish and provide official U.S. reference and standard preparations for quality assurance tests performed by the manufacturers and the Center for Biologics Evaluation and Research (CBER).

c) Coordinate the processing of protocols and the testing of product samples submitted for licensing actions or for the release of biological products manufactured under existing licenses and to issue the appropriate report of final action to the manufacturer.

In addition, the CBER continues to serve as a WHO Reference Center and DPQC is responsible for coordinating the testing and reporting of all test results to the designated authorities. Also, DPQC is the custodian of all official complaint or regulatory samples. These samples are distributed to the various testing laboratories within DPQC and the other Divisions and the results, when available, are collated and reported to the appropriate compliance personnel.

DPQC is responsible for animal care within CBER and for maintaining accreditation of the facility by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The Division currently has three veterinarians on staff of whom two are board-certified in Veterinary Pathology.

Organization and Management

Only two major changes occurred in senior staff members of DPQC during FY 92. Dr. Mark Heintzelman moved to the Division of Congressional and Public Affairs and Cdr. Clara Lin moved from the Division of Bacterial Products to the Biological Testing Laboratory, DPQC.

The current organizational structure and the incumbents in management positions are as follows:

Office of the Director
Director: Edward A. Fitzgerald, Ph.D.
Deputy Director: H. Donald Hochstein, Dr.P.H.
Biological Testing Laboratory
Director: H. Donald Hochstein, Dr.P.H.
Animal Test Section
Chief: Lawrence D'Hoostelaere, Ph.D.
Reference Reagents Section
Acting Chief: Julia M. Lukas
Pathobiology and Primatology Laboratory
Director: James H. Vickers, D.V.M.
Histology Section
Chief: James E. Hines
Animal Resources Section
Chief: John P. Cogan
Product Release Staff
Director, Deborah J. Parshall

In FY 92, we moved a major portion of the Division to the Nicholson Lane Research Center (NLRC). Only the Reference Reagents Section and the Pathobiology and Primatology Laboratory remain in Building 29A. The move was a difficult one but has given us much-needed laboratory and office space. The recently proposed reorganization of CBER will cause more changes in DPQC in FY 93. The Pathology and Primatology Laboratory will split off to become the Division of Veterinary Services while we acquire the Laboratory of Analytical Chemistry from DBB. This group, headed by Dr. Joan May, and the Reference Reagents Section of DPQC will also move to NLRC during FY 93 so that all of DPQC will be consolidated. We hope that this will allow us to function more effectively in both regulatory and research duties.

Summary of Current Research

The Division has an active applied research program concerned with improving existing quality control tests for biological products by developing new or adapting other current methodologies for product testing as required. In addition, the Division produces materials for use as official reference preparations which are unavailable from other sources, as well as participates in collaborative research projects initiated by other investigators and provides scientific management for contract-supported activities.

1. Rabies Vaccine Potency. In order to develop better means for defining the potency of rabies vaccine, three approaches are being investigated: (1) quantitation of the glycoprotein content of the vaccine by single radial immunodiffusion (SRID); (2) evaluation of the antibody-binding test in cell culture and comparison with the NIH mouse potency test; and (3) use of an ELISA assay for quantitation of rabies virus nucleoprotein and glycoprotein and the relationship of these values to classical vaccine potency.

The methodology for the SRID assay being investigated for its usefulness in potency testing rabies vaccine is the same as that employed in the potency testing of influenza vaccine strain concentrates. An antiserum to the glycoprotein of the ERA strain of rabies virus was provided by the British National Institute of Biological Standards and Control (NIBSC). Also, in collaboration with the Division of Virology, the glycoprotein from the Pittman-Moore (PM) strain, which was the origin of the seed used to manufacture the licensed HDCV strain of rabies vaccine, was isolated and purified. The purified glycoprotein was used to produce a satisfactory sheep antiserum. A comparison was made of the immunoprecipitates formed when the Reference Rabies Vaccine and several lots of licensed Rabies Vaccines were tested with the anti-PM and the anti-ERA serums. The results showed that the homologous systems gave zones which were smaller in diameter with more distinct perimeters than those obtained with the heterologous system. In collaboration with NIBSC, we have also tested an antiserum to the Flury (LEP) strain of rabies vaccine. Since Behringwerke A.G., Marburg, West Germany has submitted a License Application for Rabies Vaccine produced with the Flury strain, we will need to test this product as soon as samples are received from the manufacturer.

During FY 89, CBER received a license application from Connaught Laboratories, Ltd. (Toronto, Canada) for a rabies vaccine produced from the ERA/SAD strain of rabies virus. We have continued testing all lots of vaccine submitted in support of this application using both the NIH and SRID test procedures. This product was licensed in the second quarter of FY 92.

Comparative tests of cell culture vaccines and by both the SRID and the NIH Mouse Test, has shown the values obtained with SRID to be about 1.2 to 1.5 times that of the in vivo test. SRID continues to give more reproducible results than the NIH Mouse Test. However, the relationship between the potency values of rabies vaccine expressed by either the NIH test or SRID, and the efficacy for man has yet to be established.

We completed laboratory testing during FY 91 as a part of the most recent WHO collaborative study to compare the NIH and SRID tests. A draft report was received from NIBSC in August 1991 but the final document has not yet been issued.

The Division has continued its participation in the collaborative program with the Occupational Medical Service (NIH), to study the immunization of personnel who are at-risk of exposure to rabies virus using the cell culture Rabies Vaccine from Institut Merieux. Antibody level in immunized Division personnel are being monitored regularly to evaluate the currently recommended pre-exposure prophylaxis regimen and to determine the need for booster injections.

2. Diphtheria Antitoxin Potency. The official assay to determine the potency of diphtheria antitoxin is a neutralization

test carried out by the intradermal inoculation of rabbits. Work is in progress to develop a suitable *in vitro* assay utilizing cell cultures instead of rabbits. A suitable preparation of diphtheria toxin for routine use has been selected from among several lots evaluated and the concentration of this and the U.S. Reference Antitoxin required for the test has been established. Several different cell types, including primary rabbit kidney, human diploid (WI-38) and Vero cells have been used successfully in the microtiter system and the latter were chosen for use in this system. Good correlation between the results of the tissue culture test and the rabbit test has been obtained with the International Standard Diphtheria Antitoxin, the U.S. Standard Diphtheria Antitoxin, and the licensed Diphtheria Antitoxin, all of which were produced in horses. However, the potency of the Human Immune Serum Globulin in the cell culture assay was only one-half of the value determined with the rabbit skin test. The reason for this disparate finding is being investigated. After standard assay conditions have been defined, comparative *in vivo* and *in vitro* testing of Human Immune Serum Globulin will continue.

The potency of the diphtheria component in the DTP vaccine is currently measured by immunizing guinea pigs with the vaccine and assaying a serum pool from the immunized animals in a separate neutralization test, also performed in guinea pigs. We are evaluating the cell culture assay as a possible replacement for the guinea pig test. This would enable us to use fewer animals and also test the sera from individual immunized guinea pigs instead of using a serum pool. As with the above study, preliminary results show that potency values in the cell culture assay are lower than those in the guinea pig assay. Work is in progress to confirm and extend these findings.

3. Tetanus Antitoxin Potency. The official assay to determine the potency of the tetanus component of DTP vaccine is also an *in vivo* neutralization test using serum from immunized guinea pigs. We are trying to develop an ELISA method to determine the unit value of the serum from these animals in comparison with a reference serum. As in the above study involving diphtheria antitoxin, this would enable us to replace an expensive, variable *in vivo* assay with a cheaper, more reproducible *in vitro* assay while maintaining assurance that the DTP vaccine is potent. Preliminary work centered on the purification of tetanus toxin, selection of an in-house reference serum, calibration of the reference serum in the guinea pig assay, development of a parallel line bioassay for calculation of results and validation of the ELISA using the reference serum. We are now proceeding to test individual and pooled sera from guinea pigs immunized with DTP lots submitted for routine release.

4. Limulus Amebocyte Lysate (LAL) and Rabbit Pyrogen Test. In order to better understand the disparities between the results of the LAL tests as well as the fever response of patients to whom certain biological products are administered, a trial with the

U.S. Standard Endotoxin (Lot EC-5) was conducted in human volunteers at the Clinical Research Center in New Orleans, LA. Sixty volunteers were divided into 5 groups and given 0, 2, 4, 8 or 26 Endotoxin Units (EU) per kg by intravenous injection. Temperature was monitored every 15 minutes for eight hours. The threshold pyrogenic dose (TPD) for man in this study was approximately 4.1 EU/kg. This agrees well with other human trials and is approximately half the TPD for rabbits using EC-5 in our laboratory.

5. Molecular Biology of Poliovirus Vaccine Neurovirulence. We have significantly increased the database on neurovirulence of Sabin type 3 virus, having added data on more than 70 lots of oral polio vaccine (OPV), as well as several specially designed experimental strains and clones. The data were obtained by using our extremely sensitive molecular-biological assay of revertant particles, named MAPREC (Mutant Analysis by PCR and Restriction Enzyme Cleavage) in comparison with the data of the monkey neurovirulence safety test for polio vaccine. As a result, we have gotten the confirmation of a) strict correlation of the percentage of revertants at position 472 of Sabin type 3 genome and its neurovirulence for monkeys; b) lack of apparent effect of reversion in position 2493 of the genome on the monkey neurovirulence test; c) impact of a number of factors involved in the process of virus passage in cell culture on the rate of revertant accumulation and specification of these factors.

We performed direct sequencing of the entire genome of seven Sabin type 3 clones, chosen for elucidation of molecular determinants of neurovirulence and of three high-passage virus strains. The results of screening the genomes of the seven clones, in combination with monkey neurovirulence tests on four of these clones, gave an additional evidence that position 472 is a key determinant of neurovirulence, while 2493 is not directly linked to neurovirulence in monkeys. Screening of the high-passage virus strains revealed several other genome positions at which mutants accumulate consistently, though the rate of this accumulation is much lower than in positions 472 and 2493. This extensive study of genetic stability of OPV genomes allows us to identify unstable positions in the genome and to design a special testing procedure for vaccine consistency control.

Dr. Inessa Levenbook, DPQC, organized an International Workshop on this issue, with participation of all the leading experts in the field and over 150 scientists representing 11 countries. This was followed by a meeting of the participants of the International Collaborative Study based on MAPREC to determine all the technical and material provisions for the study.

Related Program Activities

1. Rhesus Breeding Colonies. The total ban on the exportation of wild Rhesus monkeys from India and Bangladesh remained in

effect during FY92. Consequently the CBER continued a lease agreement with the commercial manufacturer of poliovirus vaccine for the yearling progeny from one colony in return for the manufacturer paying the maintenance charges for support of the colony until the manufacturer is self-sufficient in monkey supply. This has been renewed for FY93. CBER continued to fund two additional Rhesus colonies which will meet government requirements.

The contracts for care and technical assistance for ferrets used in Influenza Vaccine testing and rabbits used for antibody production have concluded and the animals have returned to CBER for continued operation.

A new animal care contract has been initiated to provide technical and husbandry support for the CBER animal colony under the director of the CBER veterinary staff. Government animal caretakers are being reassigned and/or retrained.

2. Potency Testing of Plague Vaccine. From 1965 to 1986 the Naval Bioscience Laboratory (NBL) at Oakland, California was the FDA-designated site for the independent bioassay of the potency of Plague Vaccine, manufactured by Cutter Division, Miles Laboratories, Inc. In August, 1986, the Department of the Navy closed the NBL plague laboratory and CBER was forced to seek another site for plague vaccine potency testing. Since DPQC has the responsibility for product testing, we initiated a contract with Dr. A.E. Karu at the University of California, Berkeley to continue the <u>in vivo</u> potency testing of plague vaccine and to attempt to develop an <u>in vitro</u> assay as a replacement for the mouse potency test. Dr. Karu had data on the bioassay of six vaccine production lots measured by the standard FDA bioassay, an abbreviated bioassay using 6 mice for dilution (instead of the usual 20 mice) and the enzyme immunoasorbant assay (EIA). Reference Lot 4 was tested by all 3 assays along with 5 production lots. The result of these 5 tests indicates that the EIA results did correlate with the bioassay. However, 5 EIA/bioassay comparisons do not provide sufficient data to make a case for CBER replacing the bioassay with only the humoral potency EIA. Many more tests will be done in order to improve the correlation.

This contract will terminate in June 1993 so that Dr. Karu will not be able to continue potency testing of plague vaccine. Also, Cutter ceased production of plague vaccine but another U.S. manufacturer will possibly go into production within the next year. A decision on potency testing for CBER will have to be made at that time.

3. Lot Release System. The program for development of an automated computer-based system for coordinating the review of protocols and the reporting of the quality assurance tests performed by the CBER as well as for generating the appropriate report of final action has been described previously. All the

professional laboratory divisions are on-line and the system continues to function well. The current program no longer operates on the Data General System and has been converted to an integrated ORACLE-based system on the CBER VAX. We are also exploring the possibility of "electronic mail (E-mail)" for the transmission of the manufacturer's test data to CBER and to use E-mail to aid reviewers during their review of protocol data. The extensive use of personal computers as well as the level of sophistication of E-Mail software should allow DPQC to receive data from manufacturers and transmit this data to the reviews in other Divisions electronically. Such a program has enormous potential for speeding review and release of biological products.

4. CBER Animal Facility. The Pathobiology and Primatology Laboratory of DPQC is responsible for the care of all research animals used in CBER and for maintaining the animal facility accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Our facility will undergo its bi-annual inspection in September, 1992. At this time both the Nicholson Lane Facility and Building 29A will be inspected. We do not anticipate any problems in Building 29A but the Nicholson Lane facility is an unknown entity. We will make every attempt to keep the same standards of animal care at both locations and retain our accredited status.

HONORS AND AWARDS 1991-1992
Division of Product Quality Control

Robert G. Darius, Biologist

Group Recognition Award - FDA Desert Shield/Storm Task Force

Group Recognition Award - Acellular Pertussis Vaccine Characterization Group

Lawrence A. D'Hoostelaere, Ph.D.

Group Recognition Award - FDA Desert Shield/Storm Task Force

Evgenia Dragunsky, M.D.

Member, Inactivated Whole Cell Plus Recombinant B Subunit PLA Review Committee

Member, E. Coli/Shigella Flexneri Hybrid Vaccine EcSf 2a-2 PLA Review Committee

Scott E. Eskin, Biological Laboratory Technician

Group Recognition Award - FDA Desert Shield/Storm Task Force

Edward A. Fitzgerald, Ph.D.

Member, Committee of Revision, U.S. Pharmacopoeial Convention, 1990-1995.

Technical Advisor to World Health Organization for the replacement of the International Standard for Rabies Vaccine, 1990-1992.

Member, Center for Drugs and Biologics Task Force on Stability Guidelines.

PHS Outstanding Unit Citation, June 1992.

Marites Gabuten, Consumer Safety Officer

Group Recognition Award - FDA Desert Shield/Storm Task Force

Donald J. Gardner, D.V.M.

FDA, CBER Representative, Animal Program Advisory Committee

Member, CBER Animal Research Committee

Navy Commendation Medal

Army Commendation Medal

John W. Hagerty, Consumer Safety Technician

Group Recognition Award - FDA Desert Shield/Storm Task Force

Roy E. Henderson, Biological Laboratory Technician

Group Recognition Award - FDA Desert Shield/Storm Task Force

H. Donald Hochstein, Dr.P.H.

Project Officer, CBER Plague vaccine contract with the University of California

Member, Center for Biologics Evaluation and Animal Research Committee

Outstanding Achievement Award - For contributions in providing regulatory actions and medical products to protect the health and safety of American military in the Persian Gulf

Group Recognition Award - Acellular pertussis vaccine characterization group

Group Recognition Award - FDA Desert Shield/Storm Task Force

Inessa Levenbook, M.D., Ph.D.

Chairperson, International Workshop on Poliovirus Attenuation

Member, Publication Committee, Proceedings of the International Workshop

Member, Hybritech PLA Review Committee, CBER, FDA

Julia M. Lukas, Biologist

FDA suggestion award for preparing labels "in-house" rather than buying them for standard preparations.

Deborah J. Parshall, Consumer Safety Officer

Group Recognition Award - FDA Desert Shield/Storm Task Force

Joseph A.L. Quander, III., Biologist

Group Recognition Award - Acellular Pertussis Vaccine Characterization Group

Jeanette Ridge, Ph.D.

Member, Cytogen Corp/Cell Tech PLA Review Committee

Member, XOMA Corp PLA Review Committee

Philip J. Snoy, D.V.M.

Member, Center for Biologics Evaluation and Research Animal Research Committee

Member, Center for Biologics Evaluation and Research Clinical Research Committee

Member, Center for Biologics Evaluation and Research Chimpanzee Use Committee

Technical Advisor, NIH contract program for pest control

Project Officer, Center for Biologics Evaluation and Research Chimpanzee breeding colony

James H. Vickers, D.V.M.

FDA Representative, Interagency Research Animal Committee

Member, FDA Research Animal Council

Member, Trans NIH Coordinating Committee for Research Animal Resources

Member, NIH Animal Research Committee

Chairman, Center for Biologics Evaluation and Research, Animal Research Committee

Project Officer, CBER Rhesus Monkey Breeding Colonies

Technical Advisor to National Institute of Allergy and Infectious Diseases for contract program for hepatitis and AIDS research in primates

James H. Vickers, D.V.M. Continued

Technical Advisor to Division of Research Resources, National Institutes of Health for contract programs for primate breeding colonies

Technical Advisor to USAMRIID, Ft. Detrick, Maryland for Neurovirulence testing

Technical Advisor to Division of Pathology, WRAIR, Washington, D.C. for Neurovirulence testing

Who's Who in America 1992-1993, Biographical listing selection

Member, FDA Process Improvement Team (Contracting)

Carroll A. Wood, Consumer Safety Officer

Group Recognition Award - FDA Desert Shield/Storm Task Force

Martin J. Wright, Biologist

Group Recognition Award - FDA Desert Shield/Storm Task Force

Group Recognition Award - Acellular Pertussis Vaccine Characterization Group

PUBLICATIONS 1991-1992
Division of Product Quality Control

Konstantin Chumakov, Ph.D.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Levenbook I. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence, J Virol 1992;66:966-70.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Taffs R, Levenbook I. RNA sequence variability in attenuated poliovirus, Vaccines 92. Modern approaches to new vaccines including prevention of AIDS, Cold Spring Harbor Symp Quant Biol 1992;331-6.

Evgenia Dragunsky, M.D., Ph.D.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Levenbook I. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence, J Virol 1992;66:966-70.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Taffs R, Levenbook I. RNA sequence variability in attenuated poliovirus, Vaccines 92. Modern approaches to new vaccines including prevention of AIDS, Cold Spring Harbor Symp Quant Biol 1992;331-6.

Dragunsky E, Rivera E, Aaronson W, Dolgaya T, Hochstein HD, Habig WH, Levenbook I. Experimental evaluation of antitoxic protective effect of new cholera vaccines in mice, Vaccine 1992;10:2.

Edward A. Fitzgerald, Ph.D.

Lyng J, Weis Benton M, Ferguson M, Fitzgerald EA. International collaborative study on the fifth international standard for rabies vaccine. Submitted to biologicals for publication.

Hochstein HD, Fitzgerald EA, McMahon FG. Properties of U.S. standard endotoxin (EC-5) in human volunteers. Manuscription in preparation.

Atanasiu P, Fitzgerald EA, Kaplan MM, Koprowski H (eds). Potency test of antirabies serum and immunoglobulin. In "Laboratory Techniques in Rabies", Fourth Edition, WHO, Geneva, 1992.

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Donald J. Gardner, D.V.M.

Gibson SO, Gardner DJ, Davis JA. Ulcerative dermatitis in laboratory mice, Lab Anim 1992;20:18-2.

H. Donald Hochstein, Dr.P.H.

Dragunsky E, Rivera E, Aaronson W, Dolgaya T, Hochstein HD, Habig WH, Levenbook I. Experimental evaluation of antitoxic protective effect of new cholera vaccines in mice, Vaccine 1992;10:2.

Inessa Levenbook, M.D., Ph.D.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Levenbook I. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence, J Virol 1992;66:966-70.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Taffs R, Levenbook I. RNA sequence variability in attenuated poliovirus, Vaccines 92. Modern approaches to new vaccines including prevention of AIDS, Cold Spring Harbor Symp Quant Biol 1992;331-6.

Dragunsky E, Rivera E, Aaronson W, Dolgaya T, Hochstein HD, Habig WH, Levenbook I. Experimental evaluation of antitoxic protective effect of new cholera vaccines in mice, Vaccine 1992;10:2.

Laurie Norwood, Biologist

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Levenbook I. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence, J Virol 1992;66:966-70.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Taffs R, Levenbook I. RNA sequence variability in attenuated poliovirus, Vaccines 92. Modern approaches to new vaccines including prevention of AIDS, Cold Spring Harbor Symp Quant Biol 1992;331-6.

Monica Parker, Biologist

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Levenbook I. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence, J Virol 1992;66:966-70.

Chumakov K, Norwood L, Parker M, Dragunsky E, Ran Y, Taffs R, Levenbook I. RNA sequence variability in attenuated poliovirus, Vaccines 92. Modern approaches to new vaccines including prevention of AIDS, Cold Spring Harbor Symp Quant Biol 1992;331-6.

Philip J. Snoy, D.V.M.

Formal S, Oaks EV, Olsen RE, Wingfield-Eggleston, Snoy PJ. The effects of prior infection with virulent Shigella flexneri 2A on the resistance of monkeys to subsequent infection with Shigella sonei, J Infect Dis 1991;164:533-37.

Heyes MP, Jordon EK, Saito K, Frank JA, Snoy PJ, Markey SP and Gravell M. Relationship of neurologic status to quinolinic acid and lynurenic acid in SIV-infected macaques, J Brain Research, 1992;570(1-2):237-50.

Milenic DE, Yokota T, Fipula DR, Finkelman MAJ, Dodd SW, Wood JF, Whitlow M, Snoy P, Schlom J. Construction, binding properties, metabolism and tumor targeting of a single chain FV (sFV) derived from the pan carcinoma mAb CC49, Cancer Res 1991;51:6363-71.

James H. Vickers, D.V.M.

Heyes MP, Saito J, Markey SP, Vickers JH. Neuroactive kynurenines in brain and cerebrospinal fluid following intraspinal inoculation of rhesus macaques with live poliovirus vaccine, FASEB J 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-03001-10 OD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Comparison of in vivo and in vitro potency tests for rabies vaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Edward A. Fitzgerald, Ph.D., Director, DPQC
Others:
Nancy Roscioli, Microbiologist, DPQC
Martin J. Wright, Biologist, DPQC

COOPERATING UNITS (if any)
None

LAB/BRANCH
Office of the Director, DPQC and Biological Testing Laboratory, DPQC

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.65	0.25	0.90

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NIH mouse potency test for rabies vaccine is a highly variable assay system. We have been attempting to replace this test with a more reliable in vitro system such as the Single Radial Immunodiffusion Test (SRID) and the antibody binding test. The SRID test seems to be the most promising candidate at this time. We currently have three licensed manufacturers of rabies vaccine in the U.S. One product is adsorbed on aluminum phosphate and the other two are unabsorbed. We are testing each lot of the latter product by SRID and approximately 60% of the lots by the NIH test. The manufacturer is also testing each lot by both methods and reporting these results to us so that a statistical comparison of the two methods can be performed. The adsorbed vaccine cannot be tested by SRID, therefore, the antibody-binding test (using BHK-21 cells) and FITC-conjugated antirabies serum is used for this product in parallel with the NIH test. We also participated in a WHO collaborative study comparing the above test methods in the replacement of the International Standard Rabies Vaccine. A report for this study was presented to the WHO Expert Committee on Biological Standardization in the fall of 1991. The conclusion from this report was that the immunogenicity test (NIH) cannot be replaced by the antigenicity tests (SRID, AbB, etc.) at this time. We will continue to use SRID as a screening method with U.S. licensed rabies vaccines and explore ways to achieve better correlation between NIH and SRID tests.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-03002-11 OD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Rabies Immunization Antibody Detection in At-risk Personnel

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Edward A. Fitzgerald, Ph.D., Director, DPQC
Others:
Nancy Roscioli, M.S., Microbiologist, DPQC
Heather Hendler, Biologist, DPQC

COOPERATING UNITS (if any)
Occupational Medical Services (OMS), NIH

LAB/BRANCH
Office of the Director, DPQC

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☒ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. OBJECTIVES/METHODS: Bleed vaccinees annually; test serum for rabies-neutralizing antibody by the Rapid Fluorescent Focus Inhibition Test (RFFIT); boost those with <1.0 International Unit rabies-neutralizing antibody/ml serum with HDCS Rabies Vaccine (Institut Merieux).

2. RESULTS: Distribution of human serum quantified for rabies-neutralizing antibody in IU/ml:

Employing Agency (serum source)	Number of People Sampled	Number of RFFIT Tests
CBER-FDA	10	20
NIH	75	154
TOTAL HUMAN SERUM TESTS	85	174

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-03003-08 OD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of an SRID Potency Test for Rabies Vaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Nancy A. Roscioli, Microbiologist, Office of the Director, DPQC
Others:
Heather Hendler, Biologist, Office of the Director, DPQC
Edward A. Fitzgerald, Ph.D., Director, DPQC

COOPERATING UNITS (if any)
Division of Virology

LAB/BRANCH
Office of the Director, DPQC

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Single Radial Immunodiffusion (SRID) technique was adapted for testing rabies vaccines for potency. The glycoproteins from the Pitman-Moore (PM) and Flury-LEP strain of virus were purified and a sheep antiserum was prepared against the PM glycoprotein. We received antisera against the Flury-LEP and ERA-strains from NIBSC (London). The glycoprotein content of each vaccine strain tested was found to vary when tested using the different antisera and when compared to the International Reference Preparation (strain PM). However, if a homologous vaccine strain was used as the reference, the relative potency values remained constant. These data indicate that, ideally, the standardization of different strain of rabies virus vaccine requires a homologous reference for each vaccine strain. Vaccine lots containing PM, SAD and Flury-LEP strains were tested by SRID and compared to the manufacturers NIH results. These data were analyzed using correlation and regression analyses. The results showed that only the PM vaccines has a correlative relationship between the SRID and NIH tests. The results also suggest that, for the PM strain, the SRID it is a less variable means of determining vaccine potency. Future studies will concentrate on standardizing SAD and Flury-LEP vaccines and to correlate SRID results with immunogenicity studies for these vaccines. Accordingly, we are discontinuing this project number and merging it with Number Z01-BE-03001-10.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-03004-04 OD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of an ELISA for Rabies Vaccine Potency

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Nancy A. Roscioli, Microbiologist, Office of the Director, DPQC
Others:
Heather Hendler, Biologist, Office of the Director, DPQC
Edward A. Fitzgerald, Ph.D., Director, DPQC

COOPERATING UNITS (if any)
None

LAB/BRANCH
Office of the Director, DPQC

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An Enzyme-linked Immunosorbant Assay (ELISA) has been developed for testing rabies vaccine for potency. 96-well plates are first coated with serial dilutions of the International Reference Preparation and test vaccines, followed by incubation with the U.S. Reference Human Rabies Immune Globulin. Antigen concentration is determined after incubation with anti-human IgG coupled to peroxidase, a final incubation with substrate and optical density measurements on an ELISA plate reader with computer interface. Data analysis to determine vaccine potencies by comparison with the reference vaccine, as well as statistical significance of results, is performed using a statistical software package. Optimal working dilutions or concentrations of all reagents, including vaccines, immunoglobulin, peroxidase-linked secondary antibody and blocking agent, have been determined. Due to staff reduction in DPQC, the ELISA test has been de-emphasized in favor of the other in vitro potency tests including the Single Radial immunodiffusion Test and the Modified Antibody Binding Test. Our goal is to find a suitable in vitro test to replace the NIH test which is the current official rabies vaccine potency test. We may resume work on this test in the future pending results in other countries and recommendations by WHO concerning the potency testing of rabies vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-03005-04 OD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular assay for neurovirulent revertants in live poliovaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

PIS: Konstantin Chumakov, Ph.D., Visiting Scientist, DPQC, FDA
Inessa Levenbook, M.D., Ph.D., DPQC, FDA

Others:
Laurie Norwood, Biologist, DPQC, FDA
Evgenia Dragunsky, M.D., DPQC, FDA
Monica Parker, Biologist, DPQC, FDA
Rolf Taffs, Ph.D., DPQC, FDA
Zhengbin Lu, M.D., DPQC, FDA
Gennady Rezapkin, WHO
David Asher, M.D., NINDS, NIH
Yuxin Ran, M.D., DPQC, FDA

COOPERATING UNITS (if any)
NINDS, NIH

LAB/BRANCH
Office of the Director

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. Further confirmation of validity of our molecular-biological assay for revertants in OPV (MAPREC) was obtained by increasing database for type 3 vaccine with 65 vaccine lots from 3 other countries which demonstrated complete correlation with the results of tests in monkeys.
2. To identify a mutation(s) in the viral genome responsible for neurovirulence in monkeys, sequencing of the entire genome of seven clones of Sabin type 3 virus has been completed. A clone containing wild-type reversion at position 2493, possesses higher replication ability in vitro, but is not neurovirulent in monkey test.
3. To study mutations which may occur in poliovaccine type 3 Sabin seed virus, sequencing of the entire genome at passage level six in AGMK cells has been performed and several new mutations were found, which accumulate consistently upon passaging.
4. New extremely sensitive modification of MAPREC assay was developed, which enables to detect mutations at a level of a few hundredth of one percent and will be useful for quality control of stability of vaccine lots.
5. International Workshop was organized in December '92, attended by over 150 researchers from 11 countries, with participation of all the leading experts in the field.
6. Preparational work has been done for the WHO International Collaborative Study based on MAPREC method.
The project will continue for two more years and include types 1 and 2 poliovaccines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-03006-02 OD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mouse system for evaluation of cholera vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Evgenia Dragunsky, M.D., Ph.D., Visiting Scientist, DPQC, CBER

Others:
Inessa Levenbook, M.D., Ph.D., Research Biologist, DPQC, CBER

COOPERATING UNITS (if any)
Division of Bacterial Products

LAB/BRANCH
Office of the Director, DPQC

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments were done with a new oral killed B subunit - whole cell cholera vaccine, BS-WC, and its antitoxic protective activity was compared with live CVD 103 HgR vaccine. Intraperitoneal immunization of mice and subsequent challenge with purified cholera toxin (CT) were employed to evaluate the anti-cholera protective effect of the two new vaccines. CVD 103-HgR vaccine demonstrated 100% protection of mice against 2.25 LD_{50} and 70% against 3 LD_{50} of CT. Mice immunized with BS-WC vaccine were protected against 2.25 and 3 LD_{50} of CT in 88% and 62%, respectively. All three killed parenteral vaccines failed to protect against CT.

Based on the results we suggest the described mouse system for preliminary evaluation of the antitoxic protective activity of cholera vaccines.

Manuscript is in press.

The project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-03008-04 OD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of an in vitro assay for neurovirulence of OPV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Jeanette Ridge, Ph.D., Microbiologist, Office of the Director, DPQC
Others:
Inessa Levenbook, M.D., Ph.D., Research Biologist, Office of the Director, DPQC

COOPERATING UNITS (if any)
None

LAB/BRANCH
Office of the Director, DPQC

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
The first phase of the project is completed: methodology and procedure for advanced differentiation of neuroblastoma cells has been established and proved by three major markers analyzed by the FACS assay. The data were presented at the 1992 World Congress on Cell and Tissue Culture.

The project will continue for two more years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-01001-10 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Comparison of LAL Test with the Rabbit Pyrogen Test

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: H.D. Hochstein, Dr.P.H., Deputy Director, DPQC
Others:
N. Winpigler, Biological Laboratory Technician, Biological Testing Laboratory, DPQC
Pankaj Amin, Biologist, Biological Testing Labortory, DPQC

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biological Testing Laboratory

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Samples of lots subject to the rabbit pyrogen test submitted to the Center for Biologics Evaluation and Research for release are first testing using the LAL test. All samples with a positive lysate test at a dilution of 1:32 or greater are then tested using the standard rabbit pyrogen test (Title CFR 600.13(b)(1),(2),(3)). In addition, lots are selected in the usual fashion for the rabbit pyrogen test.

With only few exceptions, there has been a positive correlation between the rabbit pyrogen test and the LAL test.

This study will provide the basis for use of the LAL test instead of the rabbit pyrogen test in the quality control of products where appropriate.

The study will be continued in order to gather further data comparing the LAL and rabbit pyrogen test in a wide variety of biological products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 BE 01002-07 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of In Vitro Potency Assay for Diphtheria Antitoxin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Lawrence D'Hoostelaere, Ph.D., Chief, AT & QAS, BTL, DPQC, FDA
Others:
Julia M. Lukas, Biologist, BTL, DPQC, FDA
Joseph Quander, Biologist, BTL, DPQC, FDA
Edward A. Fitzgerald, Ph.D., Director, DPQC, FDA
Deborah Trout, Bio. Lab. Tech., BTL, DPQC, FDA
Nancy Roscioli, M.S., Microbiologist, DPQC, FDA
Heather Hendler, Biologist, DPQC, FDA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biological Testing Laboratory

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this study is to develop an in vitro potency assay to replace the guinea pig death test portion of the official release test for the diphtheria component. VERO cells will be used as a target for the diphtheria toxin. After screening several lot of fetal bovine serum for diphtheria antitoxin, a lot has been approved and purchased. After examining several methods for determining viable cells, the Neutral Red Assay has been selected. The U.S. Standard Diphtheria Toxin Lot 35119 will be used for the cell death assay. It is currently used in the animal death test and will be used in the rabbit skin test. A reproducible minimum cytopathic dose is being established for the 4 day assay. Inhibition of cell death will be measured using the U.S. Standard Diphtheria Antitoxin, Immune Serum Globulin, IGIV and guinea pig immune serum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-01003-04 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of an in vitro Potency Assay for Tetanus Antitoxin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Reginald Ramos, Biologist, BTL, DPQC
Others:
Lawrence D'Hoostelaere, Ph.D., Director, Animal Testing Section, BTL, DPQC
Edward A. Fitzgerald, Ph.D., Director, DPQC
Suresh Rastogi, Ph.D., Acting Director, DBE

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biological Testing Laboratory

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.0	8.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The two current ELISA standards ER2 and ER3 are labelled at 12 and 6 units, respectively. Extensive guinea pig potency testing on these standards is being performed to calculate the exact unitage to the 0.5 unit level.

ER2 is being used as the standard in the Tetanus ELISA assay to determine the titer of routine lot release serum. Sera from DTPads lots stored frozen in the past will represent one set of samples. These sera were saved, because they were borderline samples in the official potency assay. In addition samples will be collected to calculate both the individual and pool titers of each new DTPads immunized guinea pig. The pool will be tested in the official potency assay. All Tetanus ELISA assays are being performed using an automated dilutor.

A U.S. Master Standard for the Tetanus ELISA is being considered. Guinea pigs will be immunized with U.S. Standard Tetanus Toxoid Lot 1 and serum collected. The individual immune response over a 4, 6 and 8 week period is being examined using the ELISA assay. This information will give us insight into the number of guinea pigs to be used and the optimal bleed time when formulating the U.S. Master Standard. ER3 will be used for lyophilization and stability studies. Some of the variables to be considered are: 1) The starting concentration to be used in the Tetanus ELISA assay. 2) The stability of the antitoxin after being lyophilized. 3) The amount of material used to quality control the antitoxin before and after being lyophilized. 4) Pre-processing of the antitoxin before being lyophilized. 5) The type of vial and volume used. 6) The number of vials shipped per year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BE-01004-04 LBT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Diphtheria Antitoxin Master Standard Replacement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,
PI: Julia Lukas, Biologist, RRS, BTL, DPQC
Others:
Deborah Trout, Biological Laboratory Technician, RRS, BTL, DPQC
Bruce D. Lowe, Biological Laboratory Technician, RRS, BTL, DPQC

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biological Testing Laboratory, DPQC

SECTION
DPQC, CBER, FDA

INSTITUTE AND LOCATION
DPQC, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
The selection of a Diphtheria Antitoxin to serve as a replacement master standard was made. The antitoxin potency is being determined. This involves the determination of the total units per vial and the appropriateness of the preparation to serve in the following areas:

1) antitoxin potency testing in animals,
2) in vitro potency (Vero cell test in microtiter plates), and
3) rabbit skin testing

Division: Product Quality Control
Branch: Pathobiology and Primatology

OFFICE OF BIOLOGICAL PRODUCT REVIEW
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
CONTRACT REPORT
October 1, 1991 through September 30, 1992

Contractor: BIOCON, INC., FDA 223-89-1353

Title: To Provide Housing and Long Term Care for Experimental Rabbits

Date Initiated: September 25, 1989

Current Annual Funding Level: Approximately 80 rabbits are housed at the contractor's facility for an annual cost of $110,000.00.

Objectives: To provide the Center for Biologics Evaluation and Research housing and long term care for experimental rabbits. Care including feeding, weighing, bleedings and collection of biological samples and delivering them to CBER. Provides housing for approximately 75 rabbits with the possibility of increasing that amount to 150 rabbits.

Methods Employed: This colony is maintained at the BIOCON facility, 649 Lofstrand Lane, Rockville,Maryland which is AAALAC accredited.

Major Findings: None.

Significance: Due to the shortage of animal space in the CBER facilities these rabbits are being housed at a contractors facility. The contractor provides animal care as required by the DHHS Guide for the Care and Use of Laboratory Animals and performs procedures that are requested by CBER investigator.

Proposed Course of Project: This contract is ending July 1992 and rabbits will be moved to Building 29A on the NIH campus.

Division: Product Quality Control
Branch: Pathobiology and Primatology

OFFICE OF BIOLOGICAL PRODUCT REVIEW
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
CONTRACT REPORT
October 1, 1991 through September 30, 1992

Contractor: HAZLETON LABORATORIES AMERICA, INC., FDA 223-77-1104

Title: To Provide for the Year Round Outdoor Breeding and Daily Maintenance of a Colony of Rhesus (Macaca mulatta) Monkeys

Date Initiated: September 30, 1977

Current Annual Funding Level: Funding terminated July 1, 1982.

Objectives: To establish a breeding colony of Macaca mulatta monkeys that will provide 500 twelve to eighteen month old animals each year to the Office of Biological Product Review. These are to be healthy and immunologically suitable for use in biological safety and evaluation programs.

Methods Employed: Outdoor enclosures (corn cribs) are used to house these animals. The animals are placed in the "corn crib" pens in harem groups of 8 to 10 adults per pen.

Major Findings: This Rhesus monkey breeding colony and government equipment has been leased to Lederle since May 1982. Lederle will assume all costs for colony maintenance and in return will receive the young animals for vaccine tests. At the end of this leasing agreement Lederle will return a like colony in terms of age, to the government.

Proposed Course of Project: It is anticipated the leasing agreement with Lederle will continue until June 1993. Lederle expects to need the colony at least 2 more years.

Division: Product Quality Control
Branch: Pathobiology and Primatology

OFFICE OF BIOLOGICAL PRODUCT REVIEW
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
CONTRACT REPORT
October 1, 1991 through September 30, 1992

Contractor: TULANE UNIVERSITY, DELTA REGIONAL PRIMATE RESEARCH CENTER, FDA 223-91-1100

Title: Breeding and Maintenance of Rhesus Monkeys for Regulatory and Research Programs

Date Initiated: June 10, 1974

Current Annual Funding Level: This contract is funded for the current year at approximately $825,000.00.

Objectives: To maintain a Rhesus monkey breeding colony to supply approximately 500 monkeys per year to be used for vaccine testing and research by the Office of Biological Product Review.

Methods Employed: The Rhesus monkeys in this colony are currently held in half acre corrals as family groups. Twenty-two half acre corrals are located at the Delta Center. One corral contains a breeding group of African Green monkeys.

Major Findings: The total colony census is 2,050 (Rhesus) and 54 (African Greens). The colony health and production has been generally very good. There were 542 live births with a production birth rate of 70%. The group of monkeys identified with an inherited lysosomal storage disease are separated from the major colony and established as an animal model for the human disease and are on a CRADA program.

Colony Strength:

	Adult Males	Adult Females	Juveniles	Yearlings	Infants
Rhesus	389	814	298	522	27
African Greens	15	17	9	12	1

Significance: The Office of Biological Product Review currently has requirements for 1,500 Rhesus monkeys for safety testing and research per year. Further, it has become necessary to provide animals to the polio vaccine manufacturer for safety testing so that vaccine is available for the National Immunization Program. The complete ban of export of Rhesus from foreign sources continues and this domestic breeding colony is part of the

CONTRACT - Tulane University Continued

Office's program to fulfill its regulatory requirements. The colony of African Green monkeys have become important to AIDS research as 50% of the colony was positive SHTLV-III virus.

Proposed Course of Project: This is currently a cost-reimbursement contract to meet requirements. This contract was recompeted in 1991 and the incumbent contractor was awarded the contract for an additional three years.

Division: Product Quality Control
Branch: Pathobiology and Primatology

OFFICE OF BIOLOGICAL PRODUCT REVIEW
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
CONTRACT REPORT
October 1, 1991 through September 30, 1992

Contractor: LABORATORY ANIMAL BREEDING AND SERVICES, FDA 223-92-1105

Title: Establishment and Maintenance of a Breeding Colony of Rhesus Monkeys for Use in Various Research and Vaccine Testing Programs

Date Initiated: July 2, 1982

Current Annual Funding Level: This contract is incrementally funded with the current year at approximately $987,000.00.

Objectives: To re-establish and maintain a free-ranging colony of Rhesus (Macaca mulatta) monkeys on Morgan Island, South Carolina. This colony originally developed at Parguera, Puerto Rico.

Methods Employed: This colony of animals was relocated at Morgan Island, South Carolina and will continue to be free-ranging colony. Every effort was made to move and relocate these animals as intact social groups, thus minimizing disruption of this colony.

Major Findings: The total colony census is 4,197. There were 944 births with approximately an another pregnancies during 1991. The production rate (the live births/breeding females) for 1991 was 73%. There have been no significant health problems in this colony. A specific pathogen free colony has been derived from the many groups to supply animals free of Herpes B virus and Simian retroviruses.

Colony Strength:

Adult Males	Adult Females	Juveniles	Yearlings	Infant
578	1,876	599	1,048	96

Significance: The Office of Biological Product Review currently has requirements of 1,500 Rhesus monkeys for safety testing and research per year. Further, it has become necessary to provide animals to the polio vaccine manufacturer for safety testing so that vaccine is available for the National Immunization Program. The complete ban of export of Rhesus from foreign sources continues and this domestic breeding colony is part of the Office

CONTRACT - Laboratory Animal Breeding and Services Continued

of Biological Product Review's program to fulfill its regulatory requirements.

Proposed Course of Project: This contract was recompeted in 1992 and a cost reimbursement contract awarded. This colony will be asked to supply research primates to other HHS agencies since NIH has discontinued their colonies. This contract will be recompeted in late 1993.

Division: Product Quality Control
Branch: Pathobiology and Primatology

OFFICE OF BIOLOGICAL PRODUCT REVIEW
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
CONTRACT REPORT
October 1, 1991 through September 30, 1992

Contractor: New Mexico State University, Primate Research Institute, Holloman AFB, New Mexico, FDA 223-84-1004

Title: Housing and Special Care of a Breeding Colony of Chimpanzees to be used in Conjunction with Hepatitis Studies

Date Initiated: September 23, 1990

Current Annual Funding Level: This contract is funded at $390,000.00 per year.

Objectives: To maintain a chimpanzee breeding colony to supply a minimum of 10 young animals per year to be used by FDA, CDC and NIH investigators on viral hepatitis research protocols.

Methods Employed: Animals are housed in pairs in large indoor-outdoor runs. Females are individually monitored for sexual cycle and exposed to males at appropriate time. Newborns are removed from mothers at birth and raised with peers in a nursery providing 24 hour care. Some newborns are left with their mother for up to 1 year.

Major Findings: There are currently 60 females and 21 males in the contract colony. The nursery has 9 animals which have been born within the past year.

Significance: The animals produced on this contract and the financial responsibility are divided between NIH, CDC and FDA, Domestic breeding colonies are the only source of chimpanzees since they are no longer exported from Africa. The chimpanzees are used in epidemiologic and vaccine research studies of viral hepatitis including types A, B, C and non-A, non-B. Chimpanzees are the only animal model in which the human immunodeficiency virus (HIV) has caused a persistent viremia. As a result they have a significant potential for future vaccine studies in this area in which the Office of Biological Product Review will be involved.

Proposed Course of Project: This is a fixed price contract. The current 3 year contract began September 23, 1990.

SUMMARY: BIOLOGICAL TESTING LABORATORY

The Biological Testing Laboratory (BTL) continues to supply the U.S. Reference and Standard preparations for the release testing of licensed biological products. It also performs filling and freeze-drying services for other Divisions in the Center for Biologics Evaluation and Research (CBER). In addition, it performs sterility, safety and pyrogen/endotoxin testing for release of biological products and as a service for the National Institutes of Health, Clinical Center Pharmacy and Radiopharmacy. For example, over the past year we have sterility-tested at least 3000 lots of biologicals submitted for release action.

This laboratory is also responsible for storing and shipping all biological references and standards world wide. Over 1200 packages were shipped in FY 91 which contained over 18,000 pieces (ampules, vials, etc.).

SUMMARY: PATHOBIOLOGY AND PRIMATOLOGY LABORATORY

The Pathobiology and Primatology Laboratory (PPL) continues to provide a pathology service to all of the research divisions in CBER and consultation with various institutes of NIH. Research projects include those on AIDS, Hepatitis, Polysaccharide immunogenicity, Mycoplasma, Shigella, Polio and others are performed in primate facilities in Building 14D, NIH.

Animal care and use services are provided by PPL to all of the research divisions. This laboratory is also responsible for implementing the Animal Welfare Act Amendments and maintaining AAALAC accredited animal facility.

In January 1992, the newly remodeled animal facility at the Nicholson Lane Research Facility was opened and animal care was provided utilizing contract personnel under the director of CBER/PPL veterinarians.

PPL provides the project officers and technical direction on the FDA's primate breeding colonies and one chimpanzee breeding colony. PPL personnel serve as consultants to other government agencies such as NIH, DOD, CDC and VA on matters concerning veterinary pathology, vaccine testing and laboratory animal medicine.

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Mouse system for evaluation of antitoxic activity of new cholera vaccines

INVESTIGATORS: P.I.: Evgenia M. Dragunsky, M.D., Ph.D., Visiting Scientist, DPQC, CBER, FDA
Inessa S. Levenbook, M.D., Ph.D., Research Biologist, DPQC, CBER, FDA

OBJECTIVE: At the present time there are two new cholera vaccine candidates under evaluation at FDA. They are live cholera vaccine CVD 103-HgR (IND 2112) and killed whole cell-B subunit (BS-WC) cholera vaccine (INDs 3845, 32-34, 3842). In contrast to licensed current parenteral cholera vaccines the new ones are oral and content B subunit of cholera toxin (CT) to provide antitoxic immune response in people. However, there is no animal model to assess antitoxic protective effect of new cholera vaccines. The objective of the study was to evaluate antitoxic protective effect of new cholera vaccines by immunizing mice with different vaccines and subsequent challenge with CT.

METHODS EMPLOYED: Intraperitoneal immunization of mice with the two new and three old licensed vaccines; subsequent challenge with different doses of CT.

MAJOR FINDINGS: Live cholera CVD 103-HgR vaccine demonstrated 100% protection against 2.25 LD_{50} and 70% against 3 LD_{50} of CT. BS-WC vaccine protected mice against 2.25 and 3 LD_{50} of CT in 88% and 62%, respectively. All three current parenteral cholera vaccines failed to protect against CT.

SIGNIFICANCE: The described mouse system can serve as a test for preliminary evaluation of antitoxic protective activity of cholera vaccines with B subunit.

PROPOSED COURSE: This project is completed.

PUBLICATIONS: EM Dragunsky, E Rivera, W Aaronson, TM Dolgaya, HD Hochstein, WH Habig, IS Levenbook. Experimental evaluation of antitoxic protective effect of new cholera vaccines in mice, Vaccine, 1992-in press.

Poster presented at Sigma XI FDA Science Exposition 1992 entitled Interaction of Poliovirus with Human and Monkey Epithelial Cells.

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Develop of in vitro Potency Assay for Diphtheria Antitoxin.

INVESTIGATORS: PI: Lawrence D'Hoostelaere, Ph.D., Chief, AT & QAS, BTL, DPQC, FDA
Julia M. Lukas, Biologist, BTL, DPQC, FDA
Joseph Quander, Biologist, BTL, DPQC, FDA
Edward A. Fitzgerald, Ph.D., Director, DPQC, FDA
Deborah Trout, Biological Laboratory Technician, BTL, DPQC, FDA
Nancy Roscioli, M.S., Microbiologist, DPQC, FDA
Heather Hendler, Biologist, DPQC, FDA

OBJECTIVE: To develop an in vitro potency assay for diphtheria antitoxin as a replacement for the guinea pig death test and rabbit skin test.

METHODS EMPLOYED: Antitoxin will be serially diluted in a microtiter plate. Four times the minimum cytopathic dose (MCD) of toxin will be added to the wells. Antibody neutralization will be for one hour. VERO cells are added to the wells and incubated for four days. Viable cells will be determined using Neutral Red Assay.

MAJOR FINDINGS: Toxin Lot 35119 must be weighed because of variance between vials. A 10 mg/ml concentrate can be made and used for one month. A 1:1000 dilution of toxin concentrate will give four MCD for 1 x 10^4 cells per well. Although the media is not final, 2% fetal bovine serum is used. The lots of fetal bovine serum used must be tested for low antidiphtheria toxin titers. The neutral red assay examined spectrophotometrically at 540 nm gives more consistent results than media color change and is more efficient than microscopic examination.

SIGNIFICANCE: The VERO cell assay will reduce pain and suffering in animals. It will eliminate animals from some testing. The assay should be more precise and accurate than current tests. Smaller volumes of sample will be required and sensitivity should be increased.

PROPOSED COURSE: The U.S. Standard Diphtheria Antitoxin will be serially diluted and tested against four MCD of toxin. Guinea pig serum from official lot release testing will be compared using the VERO cell assay. ISG and IGIV products will be tested in the rabbit skin test and the VERO cell assay. This project will continue as long as personnel are available and promising results are obtained.

PUBLICATIONS: None

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Development of an in vitro assay for neurovirulence of OPV

INVESTIGATORS: PI: Jeanette Ridge, Ph.D., Microbiologist, OD, DPQC, FDA
Inessa Levenbook, M.D., Ph.D., Research Biologist, OD, DPQC, FDA

OBJECTIVE: To develop an in vitro assay for the study and evaluation of neurovirulence of oral poliovirus vaccines (OPV). To achieve this objective and mimic the in vivo condition in which mature spinal cord neurons are the viral targets, a human neuroblastoma cell line was selected which preliminary work showed had the capacity for differentiation.

METHODS EMPLOYED:

- cell culture techniques
- fluorescent immunohistochemical staining
- cytospin slide preparations
- fluorescent activated flow cytometry - in collaboration with Douglas A. Terle, Div. of Hematology, Lab of Cell. Hem., CBER

MAJOR FINDINGS: a) IFN, alone and in combination with NGF, induced extensive and sustained inhibition of cell growth. b) IFN, alone and in combination with NGF, induced advanced differentiation in three subpopulations of the NB cell line: neurons, Schwann cells and melanocyte precursors; this was established by the increased expression in treated cells of three protein markers specific for these cell types. c) The in vivo mouse data supports the in vitro data; tumor formation was delayed in mice receiving treated NB inoculums. d) The rate of neurovirulent reversion is affected by IFN and the stage of differentiation of the cells.

SIGNIFICANCE:

- indicates the possible therapeutic value of gamma-IFN treatment for NB tumors, (a, b, and c above).
- proved to be a useful as an in vitro method for the study of neurovirulent revertant selection of OPV in neuronal cells (d, above).

PROPOSED COURSE: Using the differentiated NB cell model to develop a 3-dimensional in vitro assay to more closely approximate the in vivo model.

PUBLICATIONS:

- the in vitro morphological differentiation and flow cytometry data was presented orally at the World Congress on Cell and Tissue Culture, June, 1992.
- a poster of this data will be presented at a Gordon Conference on: Cancer: Differentiation of Neural Cells, August, 1992

- a poster presented at the FDA Sigma XI Science Exposition 1992 entitled Gamma-IFN Induced Differentiation of Neuroblasts in the Human Neuroblastoma Clone SH-SY5Y.

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Development of an ELISA for Rabies Vaccine Potency

INVESTIGATORS: PI: Nancy Roscioli, Microbiologist, DPQC
Heather Hendler, Biologist, Office of the Director, DPQC
Edward A. Fitzgerald, Ph.D., Director, DPQC

OBJECTIVE: To develop an in vitro test for measuring Rabies Vaccine potency.

METHODS EMPLOYED: An ELISA assay was developed in 96-well plates by coating wells with dilutions of the International Rabies Vaccine Standard and test vaccines, followed by incubation with U.S. Reference Rabies Immune Globulin. Antigen concentration is determined after incubation with anti-human Ig-G coupled to peroxidose and final incubation with substrate.

MAJOR FINDINGS: Optimal working dilutions of all reagents have been determined. Due to staff reductions in DPQC, the ELISA test has been de-emphasized in favor of other in vitro potency assays.

SIGNIFICANCE: This was part of our attempt to replace the NIH potency test with a suitable "in vitro" test.

PROPOSED COURSE: Project discontinued for the present time but may be resumed at a later data.

PUBLICATIONS: None

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Comparison of *in vivo* and *in vitro* potency tests for rabies vaccine

INVESTIGATORS: PI: Edward A. Fitzgerald, Ph.D., Director, DPQC
Nancy Roscioli, Microbiologist, DPQC
Martin Wright, Biologist, DPQC

OBJECTIVE: To replace the highly variable NIH mouse potency test with a more reliable *in vitro* system.

METHODS EMPLOYED: The Single Radial Immunodiffusion Test (SRID) and antibody-binding test (AbB) were used with the three licensed rabies vaccines and results were compared with the NIH potency test values generated by the manufacturer and by CBER. Since one vaccine is adsorbed on aluminum phosphate, the SRID could not be used for this product. We also participated in a WHO collaborative study comparing these test methods in the replacement of the International Rabies Vaccine Standard.

MAJOR FINDINGS: In general, the SRID test values are higher than those found in the NIH test. Correlation coefficients between the *in vivo* and *in vitro* tests have been disappointing. The results from the collaborative study indicated that the immunogenicity test (NIH) cannot be replaced by the antigenicity tests (SRID, AbV, etc.) at this time.

SIGNIFICANCE: Use of *in vitro* test methods for control of licensed biological products should result in safer, more precise assays at lower costs.

PROPOSED COURSE: We will continue to use SRID as a screening method with U.S. licensed rabies vaccines and explore ways to achieve better correlation between NIH and SRID test.

PUBLICATIONS: None in 1992

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Development of an SRID Potency Test for Rabies Vaccine

INVESTIGATORS: PI: Nancy A. Roscioli, Microbiologist, Office of the Director, DPQC
Heather Hendler, Biologist, Office of the Director, DPQC
Edward A. Fitzgerald, Ph.D., Director, DPQC

OBJECTIVE: To adapt the Single Radial Immunodiffusion (SRID) test to the potency assay of Rabies Vaccine and compare it to the classical NIH mouse potency test.

METHODS EMPLOYED: The SRID methodology followed that of Ferguson and Schild (J. Gen. Virol., 1982, 59, 197-201). In collaboration with NIBSC (London) we developed antibody against glycoprotein from the three major strains of rabies virus currently used for vaccine production in Europe and the United States. Vaccine lots containing these strains were tested by SRID and compared to the NIH test results from CBER and vaccine manufacturers.

MAJOR FINDINGS: Test data indicate that a homolgous reference is required for the potency assay of each strain of rabies vaccine. Only the PM strain showed good correlation between the SRID and NIH tests. For all strains, the SRID test is for less variable than the NIH test.

SIGNIFICANCE: This project is part of our continuing effort to replace in vivo tests with in vitro test wherever possible. In most cases, the in vitro test is more cost effective and less variable.

PROPOSED COURSE: We are discontinuing this as a separate project and are merging it with the project report entitled Comparison of in vivo and in vitro Potency Tests for Rabies Vaccine.

PUBLICATIONS: None in 1992

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Rabies Immunization Antibody Detection in At-risk Personnel

INVESTIGATORS: PI: Edward A. Fitzgerald, Ph.D., Director, DPQC
Nancy Roscioli, M.S., Microbiologist, DPQC
Heather Hendler, Biologist, DPQC

OBJECTIVE: To test serum of at-risk personnel for antibody to rabies virus.

METHODS EMPLOYED: The Rapid Fluorescent Focus Inhibition Test (RFFIT) was used in all cases. Personnel were bled semi-annually or annually and the antibody titer determined. Anyone with less than one IU per ml was given a booster vaccination by OMS, NIH.

MAJOR FINDINGS: Eighty-five persons were tested and reports sent to OMS, NIH for review and action. All CBER personnel maintained sufficient titer to perform their assigned duties.

SIGNIFICANCE: The project monitors the protective antibody level of CBER personnel exposed (or potentially exposed) to rabies virus.

PROPOSED COURSE: This will continue in FY 93.

PUBLICATIONS: None

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Comparison of LAL Test with the Rabbit Pyrogen Test

INVESTIGATORS: PI: H.D. Hochstein, Dr.P.H., Deputy Director, DPQC
N. Winpigler, Biological Laboratory Technician, Biological Testing Laboratory, DPQC
Pankaj Amin, Biologist, Biological Testing Laboratory, DPQC

OBJECTIVE: To gather more information on the LAL test so it can replace the rabbit pyrogen test.

METHODS EMPLOYED: The LAL and rabbit pyrogen test are run together and the results are compared.

MAJOR FINDINGS: Excellent comparison between the LAL and rabbit pyrogen test for detecting endotoxin.

SIGNIFICANCE: This study provides the basis of the substitution of the LAL test for the rabbit pyrogen test for biological products.

PROPOSED COURSE: We will gather more data using both licensed biological products and experimental products from the NIH Pharmacy/Radiopharmacy to expand our database with these two tests.

PUBLICATIONS: None

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Molecular assay for neurovirulent revertants in live poliovirus

INVESTIGATORS: P.IS.: 1.Konstantin Chumakov, Ph.D., Visiting Scientist, DPQC, FDA
2.Inessa Levenbook, M.D.,Ph.D.,DPQC, FDA

Laurie Norwood, Biologist, DPQC, FDA
Evgenia Dragunsky, M.D., Ph.D., DPQC, FDA
Monica Parker, Biologist, DPQC, FDA
Rolf Taffs, Ph.D., DPQC, FDA
Yuxin Ran, M.D., DPQC, FDA
Zhengbin Lu, M.D., DPQC, FDA
Gennady Rezapkin, WHO
David Asher, M.D., NINDS, NIH

OBJECTIVE: To reveal all sites in genome of oral poliovirus vaccine (OPV) which accumulate reversions in course of vaccine manufacturing and to study the significance of these genetic changes for neurovirulence of OPV and other properties of the virus.

To determine the influence of virus growth conditions on the rate of revertant accumulation.

Knowledge of unstable sites in OPV would allow the manufacturer to control consistency of vaccine production and engineer genetically stable strains of the vaccine.

METHODS EMPLOYED: Direct sequencing and chemical mismatch cleavage study of RNA isolated from high passage preparations of OPV are used to find new mutations occurring during virus propagation in cell cultures. Quantitative assessment of these mutations is performed by mutant analysis by PRC and restriction enzyme cleavage (MAPREC). Neurovirulence of experimental lots of OPV is studied by the WHO monkey neurovirulence test.

MAJOR FINDINGS: Further confirmation of the leading role of genome position 472 in type 3 OPV was obtained, and lack of any apparent correlation between reversion at position 2493 was proved by neurovirulence testing of several clones of OPV characterized by molecular studies.

Quantitative analysis of mutants accumulation demonstrated the influence of cell substrate and culture confluence on the rate of genome reversion.

Sequencing of the entire genome of seven type 3 clones was performed. These clones were used for elucidation of molecular determinants of monkey neurovirulence. Additional evidence was obtained proving that position 472 is a key position determining

neurovirulence, while position 2493 showed no apparent relation to neurovirulence in monkeys.

Sequencing study of high-passage preparations of type 3 OPV revealed several new mutations which accumulate consistently during virus growth in cell cultures.

MAPREC for some genomic sites was improved as a result of the systematic study of its performance.

New extremely sensitive method which allows us to quantitate revertants at a level of few hundredth of one percent was developed.

SIGNIFICANCE: Extensive direct study of genetic stability of type 3 OPV is the first example of successful use of molecular methods for quality control of viral vaccines. It may become a paradigm for other types of OPV, as well as for other viral vaccines. This straightforward approach to the study of genetic stability and control of vaccine manufacturing could result in development of precise, inexpensive and reliable methods for evaluation of quality of biologicals which would be a valuable supplement and in some cases a substitute for the monkey and other animal testing.

New extremely sensitive method for mutant quantitation may be useful for assessment of prospective genetically engineered seed viruses.

Knowledge of the pattern of genetic instability of each virus vaccine would add to the understanding of virus biology and allow to engineer genetically stable strains with improved properties.

PROPOSED COURSE: The project should be continued to expand these studies to type 1 and type 2 OPV; direct sequencing of these types of OPV should be performed and study on correlation of reversions in type 1 and type 2 OPV with neurovirulence in monkeys should be completed.

Chemical mismatch cleavage study of all three types should be conducted to assure that we have revealed all the unstable genomic positions.

PUBLICATIONS: Chumakov K.M., Norwood L.P., Parker M.L., Dragunsky E.M., Ran Y., Levenbook I. (1992). RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence. Journal of Virology, 66:966-970.

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Diphtheria Antitoxin Master Standard Replacement

INVESTIGATORS: PI: Julia Lukas, Biologist, RRS, DPQC
Deborah Trout, Biological Laboratory Technician, RRS, DPQC
Bruce Lowe, Biological Laboratory Technician, RRS, DPQC

OBJECTIVE: Replace the current master standard, Lot 47A, which is about to run out, with a new master standard. The master standard is used to prepare the annual lot of US Standard (6 Units/ml) that is required by the CFR and by the Minimum Requirements (1947, 1979) for the lot release of Diphtheria vaccine. US Master Standard Diphtheria Antitoxin for Flocculation Lot F has been selected to replace Lot 47A. We are in the process of determining the potency of Lot F.

METHODS EMPLOYED AND FINDINGS: Using the Guinea pig death test defined by the Minimum Requirements, (1947, 1979), we are testing for the appropriate dilution of Diphtheria Antitoxin for flocculation Lot F that will give us 6U/ml. We have approximately determined the correct dilution and are in the process of performing confirmatory testing, using the current US Standard lot A51 (6U/ml) as the control. In FY 93, we hope to set up a collaborative study with industry to confirm our findings.

PROPOSED COURSE: This project should be completed by the end of FY93. A collaborative study is being considered.

PUBLICATIONS: None

PATENTS: None

INDIVIDUAL PROJECT REPORT 1992 ANNUAL REPORT

PROJECT: Development of an in vitro Potency Assay for Tetanus Antitoxin

INVESTIGATORS: PI: Reginald Ramos, Biologist, BTL, DPQC
Lawrence D'Hoostelaere, Ph.D., Director, ATS, BTL, DPQC
Edward A. Fitzgerald, Ph.D., Director, DPQC
Suresh Rastogi, Ph.D., Acting Director, DBE

OBJECTIVE: To develop an in vitro potency assay to replace the official in vivo potency assay.

METHODS EMPLOYED: An Enzyme Linked Immunosorbent Assay (ELISA) is being used to test guinea pig immune serum against a control serum.

MAJOR FINDINGS: A format for the ELISA assay has been established. A standard 96 well microtiter plate would contain a negative and positive control (ER3-ELISA Reference three labelled at six units/ml) and four unknown samples. The reproducibility of the assay over time was examined using control samples. The sample set consisted of 80 unknowns at six defined concentrations. Two types of tests were done on each sample in series. A rough test was done at four dilutions (1:500, 1:1,000, 1:5,000 and 1:10,000). The fine test was done using a 1:1.5 dilution series based on results from the rough test. The dilutions for the first ten plates were made using manual pipetors. The dilutions for plates eleven through twenty were made using an automated diluter. Manual dilutions showed a smaller standard deviation for five of the six concentrations tested. Reproducibility was found to be within one unit of the expected value for repeat testing.

The two current ELISA standards ER2 and ER3 labelled at 12 and six units, respectively, are being tested using the guinea pig death test to determine their concentrations at the +/-0.5 unit level. Aliquots of 50 ul were taken from the serum dilutions of both ER2 and ER3 prior to the addition of Control Tetanus Toxin T-1 and tested by the Tetanus ELISA using ER2 as a positive control. A 0.025 unit difference (0.5 unit/ml x 50 ul aliquote = 0.025 unit) in serum dilution of either ER2 or ER3 corresponds to a 0.005 unit difference in the Tetanus ELISA. According to the data, the accuracy of the serum dilutions is found to be within .003 units when tested in the Tetanus ELISA. Additional data from the Tetanus ELISA show that the U.S. Standard Tetanus Antitoxin could not be used as the control for the in vitro test. This could not be attributed to the glycerine, and probably results from differential binding of horse and guinea pig IgG to Protein G. This result means a U.S. Standard Tetanus Antitoxin must be made using guinea pigs as the immune serum source. Preliminary studies show that the anti-tetanus titer of pooled guinea pig serum plateaus between six and eight weeks following a single injection of DTP ads. (IY05). Actual unit values were from four

to 19 units during the four to ten week immunization period independent of lot. Also, individual guinea pigs will have titers that vary two to twelve fold among different lots of DTP ads at six weeks post immunization. The titers varied from seven to 24 units when tested in the Tetanus ELISA. In addition, three guinea pig serum from routine lots of DTP ads submitted for release have been tested in the Tetanus ELISA using ER2 as a control. The results from the Tetanus ELISA calculated the unitage to be > 5 units for both the pooled and unpooled serum of each of the five lots.

SIGNIFICANCE: The development of this assay as a replacement for the official *in vivo* potency assay is important with respect to the Animal Welfare Act. This will eliminate the animal death test portion of the potency assay.

PROPOSED COURSE: More correlation studies need to be performed before a decision can be made whether or not this *in vitro* assay can replace the current animal testing. The development of an ELISA Guinea Pig US Master Standard Tetanus Antitoxin will greatly aid in the progress of this assay. ER2 will be used as a standard to examine sera from DTP ads lots. Samples stored frozen in the past will be tested using the rough test/fine test format as described above. These sera represent previously tested samples which are considered to be borderline failures according to the Official Guinea Pig Death Test for Tetanus. Finally, the ELISA assay will continue to be compared to the animal death test using the pooled serum for each new lot of DTP ads submitted for official release. These ELISA's will be performed using an automated dilutor. ER3 will be used for lyophilization and stability studies. These studies will provide data to support decisions on how to produce and store the Guinea Pig ELISA US Master Standard Tetanus Antitoxin for distribution to manufacturers who perform routine tetanus testing. Some of the variables to be considered are: 1) The starting concentration to be used in the Tetanus ELISA assay. 2) The stability of the antitoxin. 3) The amount of material used to quality control the antitoxin before and after being lyophilized. 4) Pre-processing of the antitoxin before being lyophilized. 5) The type of vial and volume used. 6) The material shipped to the manufacturers for routine test. 7) The number of vials shipped per year. 8) We want a 10 to 20 year standard. The standard must be made from one batch of serum; therefore, we will need to decide the volume needed and the target unitage, before the standard is freeze-dried.

PUBLICATIONS & PATENTS: None